Project Title: Loggerhead Sea Turtles on the West Florida Shelf: Distribution, Habitat Use, and Vulnerability to Fisheries

Start and End Dates: 1 July 2013—September 2015

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PROJECT SUMMARY

This proposal seeks funding for an applied study of loggerhead sea turtle (*Caretta caretta*) distribution and habitat use on the West Florida Shelf (WFS) in the northeastern Gulf of Mexico. The WFS is a likely foraging area for large numbers of Florida loggerheads, which dominate the Northwest Atlantic Distinct Population Segment (DPS) (Conant et al. 2009). Ongoing studies of telemetered loggerheads tagged on Florida nesting beaches (Foley et al. in prep., Hardy et al. in prep.) suggest that many of these adult females occupy discrete home ranges on the WFS. Work on the WFS to date has focused on post-nesting adult female loggerheads. Our study would complete our understanding of the distribution of loggerheads on the WFS by providing new information about habitat use by and foraging behavior of subadult loggerheads (50-80 cm, straight carapace length). We would conduct Distance sampling along vessel line transects to estimate the relative abundance of loggerheads on the WFS. Loggerheads would be captured using long-handled dip nets in a manner demonstrated to be effective during our previous Section 6 grant (NA10NMF4720031). Using satellite telemetry, we would characterize the resident

foraging behavior of subadults and make comparisons to the behavior of post-nesting females described in previous work. To test the hypothesis that turtles have had long-term residency within their deep benthic foraging areas, we would analyze their diet and trophic record. Diet would be assessed through the prey contents of fecal samples; and trophic records would be analyzed from stable isotope ratios of carbon, nitrogen, and sulfur, found in samples of blood, skin and multiple carapace-scute layers. Trophic level and habitat occupancy would be determined from mixing models informed by baseline stable isotope ratios in prey animals collected as part of this study. Lastly, we would combine habitat data and relative abundance estimates to map potential spatiotemporal distribution of loggerhead sea turtles on the WFS. Loggerhead resident foraging habitats on the WFS may be characterized by deep reef, ledges or hardbottom habitats. Considerable overlap may exist among loggerhead foraging habitats and the areas fished by demersal longlines (Prytherch 1983) as evidenced by recently observed bycatch in that fishery (NMFS 2009). Data on the turtles' behavior and movements would therefore be directly applicable to fishery management decisions.

PROJECT DESCRIPTION

Study goals and objectives

The Gulf of Mexico off Florida serves as important foraging habitat for loggerhead sea turtles (*Caretta caretta*). Resident loggerheads are likely foraging among mesophotic reef (deep hardbottom) habitats on the West Florida Shelf (WFS). Turtles in these habitats are exposed to a wide variety of threats related to anthropogenic activities such as demersal longline fishing, oil exploration and extraction, persistent marine debris, and vessel strikes. The principal goal of the proposed project is to provide spatiotemporally explicit recommendations for management actions to limit mortality from these threats, by characterizing loggerhead foraging habitats and by describing the spatiotemporal distribution of sea turtles within them.

Objectives:

1) Describe the current spatiotemporal distribution, habitat use and foraging behavior of subadult (50-80 cm, straight carapace length) loggerheads on the West Florida Shelf using satellite telemetry for horizontal (tracks) and vertical (dive) movements. Analyze movements of turtles captured, telemetered, and released within waters near mesophotic reef habitat.
2) Describe habitat-use history of subadult loggerheads by analyzing their diet and trophic record. Assess prey contents of fecal samples; analyze stable isotope ratios of carbon,

nitrogen, and sulfur, found in samples of blood, skin and multiple carapace-scute layers; and model trophic level and habitat occupancy from mixing models informed by baselines of stable isotope ratios in prey animals collected from pelagic and benthic habitats where loggerheads were captured.

3) Use these new data (1 & 2 above), existing datasets, and density estimates (from vessel transect Distance sampling) to describe potential spatiotemporal intersections between loggerhead sea turtles and gulf fisheries and to recommend management actions to limit these intersections.
4) Identify benthic habitat features used by foraging loggerheads on the WFS and characterize the extent of such habitats using existing datasets and published information in order to describe the total extent of potential loggerhead foraging habitats on the WFS.
5) Identify and explain seasonal fluctuations in behavior that are relevant to the geographic extent of loggerhead foraging activity using sea temperature data collected from satellite transmitters and remote sensing platforms.

Project narrative

Management needs. The proposed study would provide information on loggerhead sea turtles in the eastern Gulf of Mexico that would guide management decisions concerning reduction of anthropogenic threats. Principal threats demonstrated for these sea turtles include effects from petroleum (Bjorndal et al. 2011), incidental catch by demersal long-line fisheries (NMFS 2009), and mortality from vessel strikes (Singel et al. 2007) and entanglement. Of these threats, the demersal longline fishery is the most spatially discrete (Hardy et al. in prep). Because take of loggerhead sea turtles by the demersal longline component of the Gulf of Mexico reef fish fishery was found by NMFS (2009) to exceed the take level by the 2005 Biological Opinion (NMFS 2005), the Service promulgated rules to reduce loggerhead exposure to the fishery. The rules included prohibition of demersal longline gear in the Gulf of Mexico shoreward of 35 fathoms east of Cape San Blas, Florida.

The WFS is a likely foraging area for large numbers of Florida loggerheads, which dominate the Northwest Atlantic Distinct Population Segment (DPS) (Conant et al. 2009). Ongoing studies of telemetered loggerheads tagged on Florida nesting beaches (Foley et al. in prep., Hardy et al. in prep.) suggest that many of these adult females occupy discrete home ranges on the WFS. Information on neritic/benthic loggerheads in the Gulf of Mexico has been limited to these telemetry studies of nesting females and to records of shore-stranded turtles. The research we propose would provide new information about habitat use by subadult loggerheads (50 - 80 cm, straight carapace length) captured from open waters of the WFS. Our observation data show that subadults are the predominate life stage among loggerheads in shelf waters (Fig. 1). Because these turtles would be immature, their movements would not be confused with reproductive migrations, and their seasonal distribution would not be biased by absence during the summer nesting season of their initial tagging period. In addition, because the loggerheads would be captured from foraging habitat on the WFS in the region historically fished by demersal longlines (Prytherch 1983), data on the turtles' behavior and movements would apply directly to fishery management decisions.

Study areas. Project study areas would be determined for the general region of the WFS where loggerhead turtles are likely to be present and where the demersal longline component of the Gulf of Mexico reef fish fishery has historically operated. For this assessment, we would turn to descriptions of historical fisheries (Moe 1963, Prytherch 1983), spatial coverages of bottom type indicating reef fish habitat (Jenkins 2011), telemetry data showing loggerhead distributions (Foley et al. in prep., Hardy et al. in prep.) and our own data on observed locations of loggerheads at sea (Fig. 1). Principal water depths would be 15 - 150 meters (Fig. 1).

Various sources of published data and work in progress on movements and home ranges underscore the importance of the WFS for foraging loggerhead sea turtles (Hart et al 2011, Foley et al, Hardy et al in prep). We will use these studies to focus our search efforts. However, none of this work has focused on life stages other than adult females encountered on nesting beaches. Observations of loggerheads made during aerial surveys provide some additional descriptions of spatiotemporal distribution and will also be employed to guide search efforts (e.g., Fritts et al. 1983, Griffin and Griffin 2003). Anecdotal observations of loggerheads in this area indicate that individuals may inhabit discrete natural or anthropogenic benthic habitats (Rosman et al. 1987 or hydrothermal springs (Fanning et al. 1981). Known positions of such discrete habitat features will also inform how we target search areas.

Methods. We propose direct sampling of loggerheads in the open waters of the WFS, Gulf of Mexico. Although captures of turtles in deep-water habitats are difficult, we propose a cost-efficient study using an existing Florida Fish and Wildlife Research vessel as the primary means of capture. In a pilot effort to capture subadult loggerheads at sea in this region, our methods have been successful (e.g., two captures and transmitter attachments in one day) (Fig. 2).

Our principal research vessel would be an 8.2 meter power catamaran. We would conduct approximately 5 two-day vessel trips annually (~15 trips total) out of ports along the west-central coast of Florida. Primary ports of departure would be Sarasota, Marco Island and St. Petersburg. On vessel cruises we would conduct line transects at targeted study sites on the WFS. Observations of all sea turtles would be recorded using Distance sampling methods (Buckland et al. 1993). Because vessel speeds along transects would be approximately 15 knots, a typical day with six hours of search time (not including an additional 6 hours of transit time) would be 90 nautical miles. During transects, two observers would search for turtles from a tower on the vessel (2.3 m above waterline) while a third crewmember would record vessel path and observation locations by WAAS GPS. Observation data would include each turtle's perpendicular distance from the transect line as measured by a digital inclinometer, which would provide a sighting angle for the trigonometric distance function. Estimation of turtle density would be modeled using DISTANCE 6.0 using the transected study area, frequencies of observation, and distance of turtles from transects. A limited number of overnight trips would also be conducted aboard chartered sportfishing vessels (12-20 m length, twin inboard). The purpose of the limited number of overnight trips is to allow more efficient access to deeper study sites by minimizing travel time. Overnight trips will also be paired with benthic prey item sample collection dives.

A subsample of subadult loggerheads (no more than 100 annually) would be captured using a customized long-handle dip net (Figs. 3, 4 and 5). In past efforts, we have been successful in safely capturing these loggerheads by approaching them as they bask or as they linger at the surface during dive recovery (Fig. 3). Captured turtles would be weighed and measured, and given external metal and internal PIT tags.

Approximately 30 subadult loggerheads would be selected to receive satellite transmitters during the first two years of the project (no more than 15 per year). This sample size would provide adequate estimates of home range and foraging site fidelity for management use, within what is possible given our budgets for satellite transmitters and Argos air time. Prior to transmitter attachment, epibiota would be removed and the carapace would be cleaned with water and a mild detergent. Transmitters would be applied to the carapace at the flattest portion with preference to position 3 (vertebral 3) as described in Jones et al. (2011). Transmitters would be applied using fast-setting epoxy (T-308, Powers Fasteners, or similar) as described in Mansfield et al. (2009).

Satellite transmitters would be equipped with GPS receivers, depth and water temperature sensors. Preferred transmitters are from wildlife computers, SPLASH10-F-400, Rectangle Backmount, 238A, 102mm by 56mm by 30mm, 225g. The units would be programmed to collect data at spatial and temporal resolutions that are sufficient to capture daily, seasonal and annual changes in behavior. Sensor data would be transmitted through the Argos system, analyzed biweekly and stored on a local server. Spatial patterns in behavior would be examined using the GPS positions and dive depth information. All telemetry data would be stored within a spatial database.

Through collaborations with colleagues at the Gulf of Mexico Fisheries Management Council and the University of South Florida's College of Marine Science, we will provide detailed characterizations the WFS habitats used by loggerheads. Bathymetric information would be attributed to location data in order to describe dives relative to bottom depth. Mesophotic reef habitats relevant to the distribution of loggerheads will be identified and described. We would examine sea surface temperature and ocean color data in order to characterize seasonal changes in environmental conditions as they relate to the distribution of loggerheads. The temperature data collected from satellite transmitters would be used to describe the detailed seasonal temperature regimes encountered by tracked animals and ground-truth the remotely-sensed data.

We would describe longer term habitat-use history of subadult loggerheads by analyzing their diet and trophic record. Diet would be determined by examining fecal samples opportunistically collected (volunteered, not taken) while turtles are on board during measurements and transmitter attachment. We anticipate a fecal sample from no more than 100 captured turtles per year. Samples would be stored in 70% isopropanol. In the laboratory, fecal material would be identified to lowest possible taxon by use of a dissecting microscope, and where possible, categories of material would be dried in an oven and weighed.

To assess the trophic record of captured loggerheads, we would analyze stable isotope ratios of carbon, nitrogen, and sulfur, found in samples of blood, skin and multiple carapace-scute layers. These different tissues would provide a record of stable isotope ratios ranging from days (in blood serum) to years (in the oldest carapace scute layers). Intervening ratios would allow us to determine whether the turtle had experienced major changes in habitat or diet. Blood for stable isotope analysis would come from 7-ml samples (for turtles no smaller than 50 cm SCL) drawn from the turtles' cervical sinus. The collected blood would be spun with a centrifuge for 10 minutes. Serum would be collected by a disposable pipette and kept in liquid nitrogen. The skin and carapace scute samples would be collected using a 6 mm diameter biopsy punch. The samples would be wrapped in aluminum foil and stored in liquid nitrogen. In the laboratory, carapace scute samples would be divided between eight layers, which would receive separate analyses of stable isotope ratios. Stable isotope samples would be transferred to Simona Ceriani of the University of Central Florida.

We would model trophic level and habitat occupancy from mixing models informed by baselines of stable isotope ratios in prey animals collected from pelagic and benthic habitats where loggerheads were captured. To sample prey items, identify from fecal contents. Two scuba divers would collect prey items during night dives. Five different individuals of same species among five taxa (species, groups) would be analyzed for carbon, nitrogen, and sulphur stable isotopic signatures.

Stable isotope samples would be prepared following the standard procedures (summarized by Reich and Seminoff 2010). The top layer of the skin (stratum corneum) will be separated from the underlying tissue (stratum germinativum), rinsed with deionized (DI) water and finely diced. The surface of the scute samples will be cleaned with an alcohol swab and rinsed with DI water. Prepped skin and scute samples will be dried a 60° C for 24 hours (h). Plasma and RBCs will be freeze-dried for 48 h before being homogenized with mortar and pestle. Lipids will be removed from a sub-sample of each tissue using a Soxhlet apparatus with petroleum ether as solvent for 12 h and placed in a dry oven overnight to remove any excess of solvent. In order to reconstruct the turtle dietary history, we will use a micromill New Wave with a *x, y* and *z* axes control to a precision of 1 μm. Scutes will be micro-sampled longitudinally in 50 μm layers to provide sufficient samples for stable isotope analysis.

Prey samples will be defrosted, washed, cleaned, grinded and re-frozen. Preparation will vary depending on the sample (e.g. mollusks will be removed from their shell, crustacean will be removed from the exoskeleton, jellyfish will be grinded whole). Prepped samples will be freeze-dried for 3-5 days before being homogenized. Lipids will be removed from a sub-sample of each prey item using a Soxhlet apparatus with petroleum ether as solvent for 24 h and placed in a dry oven overnight to remove any excess of solvent.

Sub-samples of prepared turtle tissues and prey items will be weighed with a microbalance and packed in tin capsules. Prepared samples will be sent to the Stable Isotope Core Laboratory at Washington State University (Benjamin Harlow--Lab Manager) where they will be analyzed for stable carbon, nitrogen and sulfur isotope ratios (^{13}C, ^{15}N and ^{34}S) using a continuous flow isotope ratio mass spectrometer (EA-IRMS).

We will use the isotope mixing model implemented in SIAR (Parnell et al 2010) to estimate the proportional contribution of prey to the diets of the turtles sampled and infer what proportion of the turtle diet is made up of organisms associated with the mesophotic reef habitat vs. elsewhere. To make inferences about a consumer's diet from its tissue, estimates of discrimination factors are required. We will use the discrimination factors that are currently developed by Ceriani et al. in sub-adult and adult loggerheads.

A subsample of turtle skin samples would be used in a compound specific stable isotope analysis of amino acids (CSIA-AA). This analysis has benefits beyond bulk tissue analyses because some amino acids differ predictably in how they retain nitrogen isotopes from primary production. This allows us to obtain trophic information without a complete sampling of the turtle’s prey items. For this analysis, Tissue samples from a sub-sample of satellite-tracked loggerheads will be sent to the University of Hawaii (Honolulu) where they will be prepared for CSIA-AA using standard protocol (Seminoff et al. 2012).

To determine the isotopic niche of the loggerheads we sample, we will analyze the variation in ^{15}N, ^{13}C and ^{34}S in scute serial layers using MANOVA with the Wilks' lambda test and then use protected ANOVAs to compare variation in isotope ratios within and among turtles.

The fractional trophic position of loggerheads samples will be calculated using the measure δ^{15}N values of glutamic acid and phenylalanine (Chikaraishi et al. 2009). In samples of consumer tissues phenylalanine appears to retain the isotopic composition of nitrogen sources at the base of the food web, whereas glutamic acid is significantly enriched in ^{15}N as it moves through the food web.

Benefits or results expected

Information on foraging loggerheads of the West Florida Shelf would guide regional management of this population. Concern for this population has recently increased due to bycatch in reef-fish fisheries (SEFSC 2008) and a decline in nesting females (Witherington et al. 2009). The proposed study targets areas of the WFS having such potential threats to loggerheads as: bottom long-line fisheries, shrimp trawling, offshore aquaculture, alternative energy production, offshore oil and natural gas platform development, and commercial vessel traffic. The identification of discrete foraging areas in this area would also provide a strong foundation for additional research studying this segment of the population. An assessment of the distribution, habitat use and movements would refine estimates of the level of threats to adult and sub-adult loggerheads on the WFS.

Each objective of this proposal would generate management relevant results that are critical for the recovery of North Atlantic sea turtles, particularly the loggerhead. Our research objectives address two of 13 recovery objectives of the loggerhead recovery plan (NMFS &USFWS 2008):

10. Minimize bycatch in domestic and international commercial and artisanal fisheries.
11. Minimize trophic changes from fishery harvest and habitat alteration.

Our research objectives address several recovery actions of the loggerhead recovery plan (NMFS & USFWS 2008):

14. Determine geographic distribution patterns of in-water populations.
    141. Develop and maintain a comprehensive GIS database of distribution and abundance.
    142. Determine migratory pathways for all life history stages.
271. Assess, categorize, and map neritic habitats used by loggerheads.
29. Develop and maintain a comprehensive GIS database of neritic and oceanic habitats (used by loggerheads) and human activities that impact these habitats.
624. Minimize loggerhead bycatch in domestic commercial pelagic and demersal
longline fisheries.
    6242. Integrate information gathered in 6241 with turtle distribution data (linked to actions 141 and 29).

6245. Investigate the effectiveness of time-area closures to minimize loggerhead interactions in domestic commercial pelagic and demersal longline fisheries.

Dissemination of information

FWC would make data summaries and maps available to other researchers, publish finished analyses, and disseminate syntheses of results and conclusions through presentations at scientific conferences, inter-agency meetings, the Gulf of Mexico Fisheries Management Council, and web postings. Summary findings of this project would be provided annually to the inwater research database making results immediately accessible to managers and other researchers. Specific examples of data sharing and outreach include near-real-time posting of satellite telemetry data on seaturtle.org, data cooperation with agency status-review efforts, presentation at the annual International Sea Turtle Symposium, posting on the FWRI website (http://research.myfwc.com/), and submittal of multiple articles to refereed journals. Data collected would also be made available to the FWRI Outreach department for educational and informational displays and classroom exercises.

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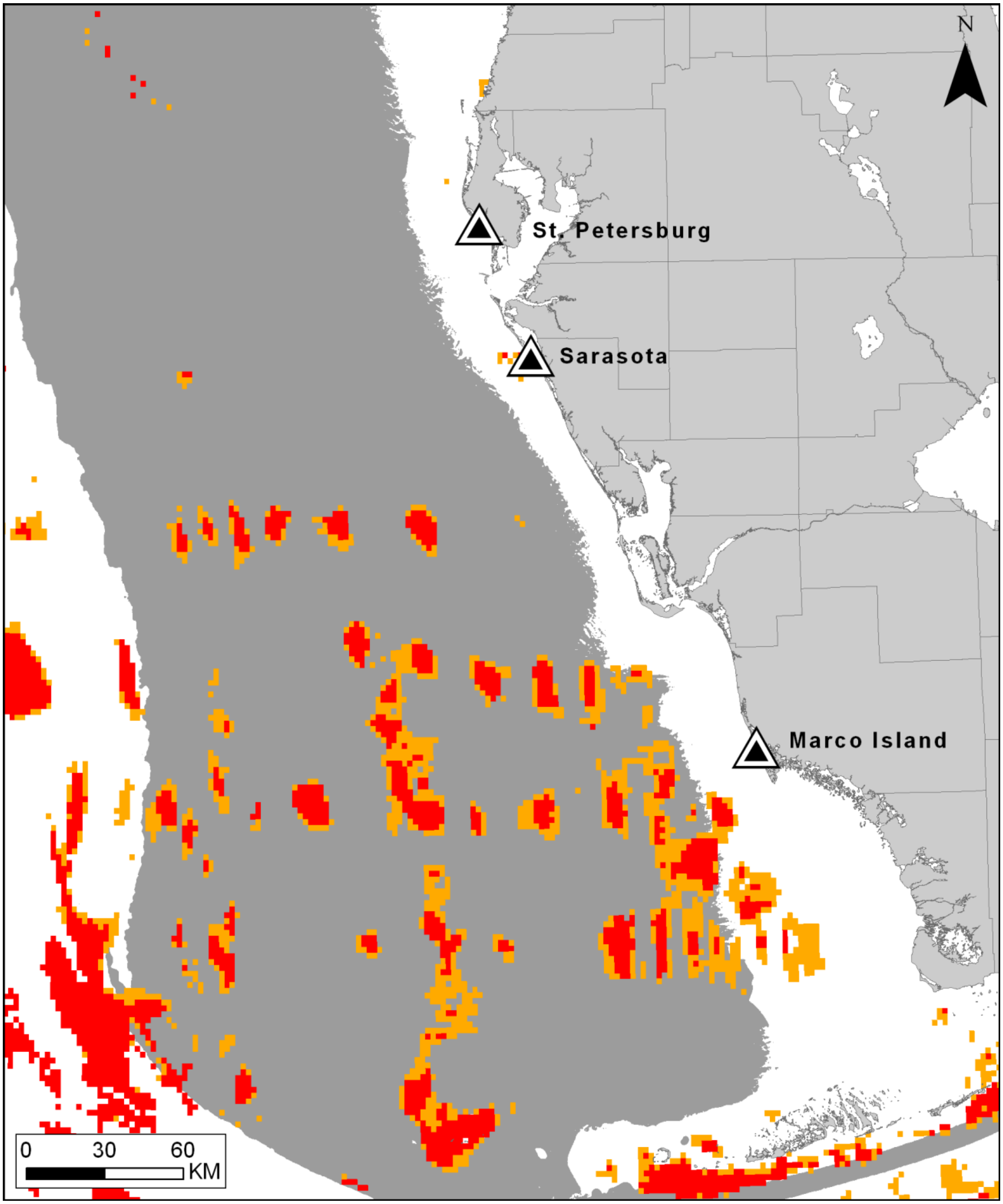

Figure 1. Potential search targets on the WFS identified as being within the 15 and 150 meter depth ranges (shaded region) and based on the presence of rock bottom types (red and orange regions)(Jenkins 2011).

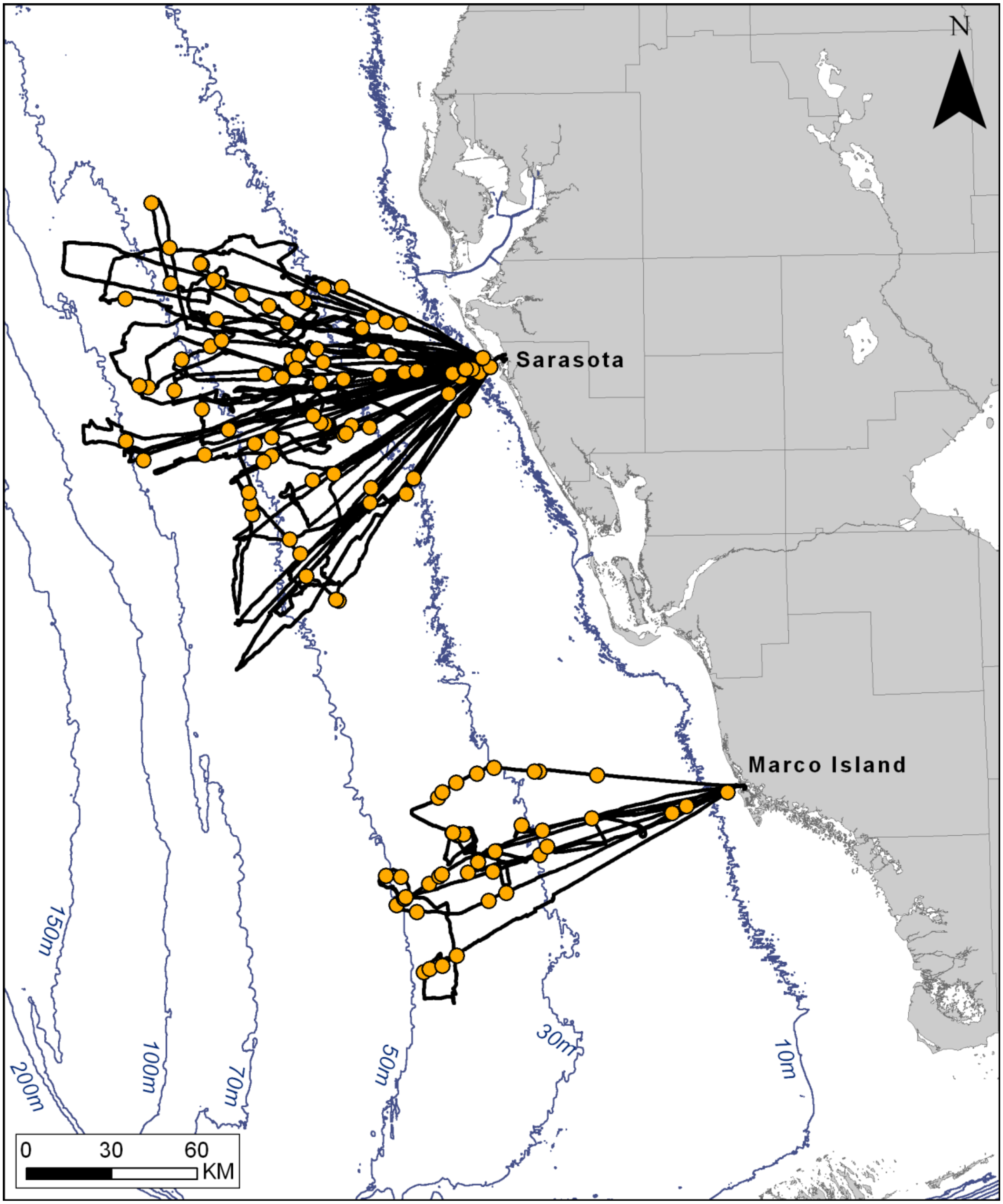

Figure 2. Vessel transect paths (black lines) and subadult or adult loggerhead observations (orange circles) collected on the West Florida Shelf during trips made from 2005 through present.

Figure 3. Example of surface-basking adult loggerhead in the Gulf of Mexico observed at offshore from Sarasota, Florida in 2009.

Figure 4. A long-handled dip net used to capture loggerhead sea turtles at the surface in waters of the West Florida Shelf.

Figure 5. A captured loggerhead sea turtle (75 cm straight carapace length) brought to the boat. The dip net's hoop detaches to allow easier hoisting of the turtle from the water into the vessel.

Project Title: Surface-pelagic juvenile turtles in oceanic waters of the Gulf of Mexico: A focus on the Gulf Loop Current and associated eddies

Start and End Dates: 1 July 2013—September 2015

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PROJECT SUMMARY

This project would fill gaps in information on the distribution, movements, trophic ecology, and vulnerability of surface-pelagic juvenile sea turtles in the Gulf of Mexico. Previous work in this region (Witherington et al. 2012) has focused on turtles outside the Gulf's largest oceanographic features—the Gulf Loop Current (GLC) and associated eddies. We propose to survey habitat identified by pelagic *Sargassum* at major convergence zones associated with GLC features in oceanic waters of the western, central, and eastern Gulf. The work would benefit conservation of four sea turtle species: loggerheads (*Caretta caretta*), hawksbills (*Eretmochelys imbricata*), green turtles (*Chelonia mydas*), and Kemp's ridleys (*Lepidochelys kempii*). Benefits would include habitat mapping, which would better inform decisions about potentially disruptive activities such as alternative energy and fossil fuel development, *Sargassum* harvest, and military maneuvers. The work would also guide response/rescue of pelagic sea

turtles following oil spills. Additional benefits include identification of genetic stocks and estimation of somatic growth. We would access *Sargassum* habitat at major convergence zones 180–300 km offshore from ports in Texas, Louisiana, and central Florida. There, we would conduct transect sampling in lines of *Sargassum*, record all turtle observations including behavior and habitat associations, and opportunistically capture turtles for additional study. We would record diet items in esophageal lavage and fecal samples; collect blood, skin, and carapace scute samples for future stable isotope analysis of trophic history, and transmitter a subsample of turtles with miniature satellite tags to record post-release movements and habitat use. Satellite-track movements and habitat observations would provide verification of remotely sensed data for *Sargassum* habitat mapping. We would use existing and new data on habitat use to map potential spatiotemporal distributions.

ACCOMPLISHMENTS OF RELATED SPECIES RECOVERY GRANT PROJECTS

The proposed work builds upon two previously funded efforts to study the biology and conservation needs of surface-pelagic neonate and juvenile turtles: Pelagic-stage sea turtles in the eastern Gulf of Mexico (funded 2009) and Florida Marine Turtle Research and Conservation Program (funded 2010). We published a synthesis of this work (Witherington et al. 2012), which set a foundation as the first direct study of sea turtles within the pelagic *Sargassum* community. Our results support a description of pelagic *Sargassum* as a transient hot spot for young sea turtles and a focal point for threats including debris ingestion and petroleum.

PROJECT DESCRIPTION

Study goals and objectives

Our study objectives apply toward mapping the spatiotemporal distribution of surface-pelagic turtles and their habitat in the Gulf of Mexico.

Objectives:

1. Conduct line transect sampling to estimate densities of surface-pelagic neonate and juvenile turtles from representative locations at major oceanographic features in the eastern, central, and western Gulf of Mexico. Describe turtle behavior and associations with surface features.
2. Capture surface-pelagic neonate and juvenile turtles to determine size, weight, injuries, and evidence of tar and plastics ingestion. Determine mtDNA haplotypes from skin biopsy samples.

3. Analyze movements of no more than 60 surface-pelagic juvenile sea turtles (annually, among 4 species) transmittered with Argos-linked satellite tags following their capture in pelagic *Sargassum* habitat.
4. For surface-pelagic neonate and juvenile turtles in the Gulf of Mexico, conduct analyses of diet and habitat use by assessing esophageal lavage and fecal samples.
5. Assemble remote sensing and ground-truth observations within a spatiotemporal map of potential pelagic *Sargassum* habitat in the Gulf of Mexico.
6. Map the estimated abundance and distribution of surface-pelagic juvenile turtles in the Gulf of Mexico based on potential pelagic *Sargassum* habitat and the behavior and movements of observed and telemetered turtles. Describe oceanographic factors correlated with the formation, persistence, movement, and degeneration of surface-pelagic turtle habitats, and with the potential concentration of hazards such as plastics and petroleum.
7. Recommend response/rescue protocols for surface pelagic turtles affected by oil spills.

Project narrative

<u>Management needs and permitting</u>

Our requested permit additions would allow additional ecological understanding and more powerful hypothesis testing for a more complete description of the distribution, movements, trophic ecology, and vulnerability of surface-pelagic juvenile sea turtles in the Gulf of Mexico. Take numbers requested represent a balance between statistical power and budget limits. Additional activities (principally, tissue sampling for stable isotopes) would allow broader conclusions about trophic ecology than ongoing diet studies allow. Previous work on pelagic juvenile turtles in the region (Witherington et al. 2012) has focused on turtles outside the Gulf's largest oceanographic features—the Gulf Loop Current (GLC) and its associated mesoscale eddies. The new work would survey habitat identified by pelagic *Sargassum* at major convergence zones associated with GLC features in oceanic waters of the western, central, and eastern Gulf (Figs. 1 & 2). The proposed work would benefit conservation of four sea turtle species: loggerheads (*Caretta caretta*), hawksbills (*Eretmochelys imbricata*), green turtles (*Chelonia mydas*), and Kemp's ridleys (*Lepidochelys kempii*). Conservation benefits would include sea turtle resource mapping, which would better inform management decisions about potentially disruptive activities such as oceanic alternative energy development, *Sargassum* harvest, military maneuvers, and oil and gas exploration/extraction. The work would also guide rescue of pelagic sea turtles during oil spill response efforts. Additional benefits include identification of genetic stocks and the estimation of vital rates such as somatic growth, information needed for demographic modeling and status assessments.

Study areas

Project study areas would be determined by the locations of oceanographic features accessible by chartered sportfishing vessels operating from three principal gulf ports located in Texas, Louisiana, and central Florida (Fig. 1). Sampling locations would be 180–300 km from port at major convergence zones rimming the GLC and associated eddies (Fig. 2). We would identify coarse locations from remotely sensed sea surface temperature, color, and current data. Specific locations would be identified in situ from visual appraisals of pelagic *Sargassum* density.

Methods

Search transects—Surface-pelagic juvenile sea turtles and their habitat would be accessed by means of chartered 14–20 m sportfishing vessels. To accommodate extended work at the remote locations, trips would be for 3 day intervals. Offshore work would be conducted during calm sea periods from May through September. We anticipate one multi-day cruise from each of three ports each year for a total of approximately 27 sampling days over three years.

During offshore trips we would conduct turtle-search transects through lines of consolidated floating material (*Sargassum* drift habitat). Transects would be timed, and vessel tracks would be recorded by WAAS GPS. Two observers would search for turtles from the vessel's tower (4–6 m above water) while a third crew member monitors observations along the vessel trackline and records observation data from all observers. Observation data would include the vertical angle of turtle's location from the observer's eye, as measured by a digital inclinometer. Perpendicular distance between the transect line and each turtle would be calculated by trigonometric function. We would use the program Distance 6.0 to estimate detectability functions, effective transect widths, and turtle density. Turtle observations would also include turtle behavior and habitat associations.

Turtle captures and sample collection—In addition to recording observations, we would capture turtles with long-handled dip nets (Fig. 3). We would employ methods we have used to safely capture 1464 surface-pelagic turtles of four species (1369 post-hatchlings, 95 juveniles) from an 8.5 m vessel (Witherington et al. 2012) and to safely capture hundreds of additional juveniles from a variety of sportfishing vessels like those we propose to charter. Given our proposed sampling effort and capture success rate, we approximate that we would capture no more than the numbers by species described in the take table during the course of this study. However, our capture rates in major convergence zones may be much higher than rates experienced on previous transects through minor convergences.

Each captured turtle would be kept moist in the shade during sampling. For every turtle captured, we will record: 1) behavior at first observation, 2) turtle location relative to floating objects, 3) health condition, 4) carapace length, 5) weight, and 6) evidence of plastic and tar ingestion from mouth examinations. Following aseptic techniques, captured turtles larger than 10 cm straight carapace-length would be tagged with passive integrated transponders (PIT tags) in the front flipper. Following aseptic techniques, we would use a 4-mm biopsy punch to collect a skin biopsy of the trailing edge of the rear flipper to conduct genetic analyses using mitochondrial DNA (mtDNA) sequencing. Skin samples would be stored in 95% ethanol. Haplotype data generated in this study, along with publicly available haplotype data from green turtle nesting populations, would be used to estimate contributions by nesting populations to the pelagic mixed stocks. Although the principal focus of this study would be on green turtles, samples from hawksbills and loggerheads larger than 8 cm SCL would also be collected. Genetic samples would be transferred to Brian Shamblin of the Marine Turtle Genetics Program, NOAA-NMFS Southwest Fisheries Science Center.

Our sampling would include collection of diet items from esophageal lavage and fecal samples. Turtles greater than 5 cm SCL would be sampled using the methods outlined by Forbes (1999). Some of our proposed lavage techniques, more specific than those outlined by Forbes, include provisions for using the procedure with small turtles (Witherington 2002). These techniques include use of small-diameter (3–10 mm) silicone tubing, and use of a hand-pumped rubber bulb in lieu of a larger (overly powerful) stomach pump. We will also collect fecal samples opportunistically. Turtles often defecate following the gastrocolic response provided by the esophageal lavage. All diet items would be stored in 70% ethanol.

We would also collect skin and carapace scute samples for trophic analyses from stable isotope ratios. Sampled turtles would be > 10 cm straight carapace-length. We would use aseptic techniques with a 6-mm biopsy punch to collect a skin sample from the shoulder and to collect a scute punch from the thickest portion of a first or second costal scute. This scute punch would provide three layers of material. The skin and scute samples would comprise as many as four representative time periods of trophic history from recent experience (skin) to the turtle's post-hatchling period (exterior scute). The collected samples would be wrapped with aluminum foil, stored in a liquid nitrogen tank, and banked until future funding allows analyses of stable carbon, nitrogen, and sulfur isotope ratios (^{13}C, ^{15}N and ^{34}S). For future baselines of stable isotope ratios in prey organisms, we would also collect a variety of prey items surrounding captured surface-pelagic juvenile turtles. Prey items would be frozen on board the vessel and later banked in a -20 C freezer. We would collect samples from eight species per year, five specimens per species, and for three years of the project (120 samples). Prey species and species groups would be selected using information from previous and proposed diet samples and would include *Sargassum*, hydroids, mollusks, crabs, bryozoans, polychaetes, and insect carrion (Witherington et al. 2012). Stable isotope samples would be transferred to Simona Ceriani of the University of Central Florida.

Diet—Items collected from lavage and fecal samples would be washed, strained, and identified under microscopy to the lowest possible taxon. Special attention would be placed on the identification and categorization of marine debris. In the laboratory, fecal and lavage sample items would be identified to the lowest possible taxon or object category by use of a dissecting microscope. Separate taxonomic groups and categories of material would be dried in an oven and weighed.

Post-capture movements—We would attach miniature Argos satellite transmitters (PTTs) to a subsample of no more than 60 (per year) juvenile sea turtles >15 cm SCL captured within surface-pelagic habitat. The PTTs would be 9.5 g, solar powered tags, attached with "soft" adhesive using the methods of Mansfield et al. (2012). The attachment uses a flexible adhesive to hold the tag fast. Attachment duration is expected to be less than 6 months, after which the tag would fall off (Mansfield et al., 2012). Even smaller, more subject-ergonomic and hydrodynamic tags would be used as new developments allow. We will be working collaboratively with Kate Mansfield (Florida International University) on innovative tag development and best available satellite tracking technology. An annual distribution of telemetered turtles would not surpass those totals described in the take table, distributed between the three study regions of the western, central, and eastern Gulf of Mexico. The species ratio is similar to the species occurrence in the eastern Gulf of Mexico (Witherington et al. 2012). Raw Argos location estimates would be subjected to plausibility filtering based on speed, angle and geometry using a filtering program created by Douglas Argos Filter Algorithm (Douglas 2006). These data would be used to assess sea turtle movements relative to drift habitat and surface advection identified using satellite imagery. In work under the previous NMFS Section 6 project grant, we successfully tracked 10 surface-pelagic Kemp's ridleys using the proposed methodology. The tracks included one path between pelagic and coastal habitats indicating a developmental transition.

Habitat detection and quantification—We would identify pelagic drift habitat using MODIS and Landsat satellite imagery in collaboration with Dr. Chuanmin Hu of the Optical Oceanography Laboratory at the University of South Florida College of Marine Science. Dr. Hu has developed an algorithm (Floating Algae Index) designed to detect floating algae using Landsat imagery (Hu 2009). Satellite imagery would be obtained at no cost from the USGS and NASA. Temporally relevant imagery would be analyzed for presence of floating algae. These features would be digitized and their areas quantified to complete a GIS of pelagic sea turtle habitat.

Pelagic drift habitat would be quantified based on the areal estimates from analyses of remotely-sensed imagery. We would record *Sargassum* habitat patch sizes during vessel transects which would serve to refine our estimates of habitat from remotely-sensed platforms. When possible, vessel transects would be timed to coincide with Landsat overpasses in order to provide coincident in situ and remotely-sensed data. Information collected during these trips would advise surface pelagic habitat estimates as well as our ability to identify habitat features in near-real time.

An end product of this research would be a set of maps describing the spatiotemporal distribution of surface-pelagic juvenile turtle habitat in the Gulf of Mexico. We would also present a useful trophic description for these turtles and describe prey species. We also anticipate estimation of growth rates and expect to have either modeled or empirical evidence for patterns of recruitment to coastal habitats.

Data sharing

Summary findings of this project would be made available to researchers and managers through annual updates to the in-water research database. Pending funding, the Florida Fish and Wildlife Conservation Commission plans to make this database publicly available through an online in-water sea turtle research and monitoring information system, which would contain management-relevant information on regional in-water sea turtle occurrence and distribution. Details of this data sharing plan are outlined within our associated proposal, "In-water sea turtle research and monitoring inventory: connecting managers and research results."

Outreach and education

We would use the results of the proposed project to conduct variously targeted forms of public outreach and education. Our efforts would be similar to what we have done as part of two related NMFS Section 6 Species Recovery Grant projects. In 2011 and 2012, we made three oral presentations and two poster presentations at scientific meetings on subjects of surface-pelagic turtles, their threats, and their ecology. We also published a paper on our results in a peer-reviewed journal (Witherington et al. 2012). In addition, we participated in two public lectures and assisted in the production of a poster describing the biology of the pelagic *Sargassum* community. Approximately 1000 copies of the poster have been distributed to schools, nature centers, and other public venues. As in previous work, data summaries and maps from the proposed work would be made available to other researchers and near-real-time satellite telemetry positions would be posted on seaturtle.org. Additional summaries would be posted on the Florida Fish and Wildlife Research Institute website (http://research.myfwc.com/) and be exported by the FWRI Outreach department for educational displays and classroom exercises.

Benefits or results expected

The proposed work would benefit conservation of four sea turtle species: loggerheads, hawksbills, green turtles, and Kemp's ridleys. Conservation benefits would include sea turtle resource mapping, which would allow informed management decisions about potentially disruptive activities such as oceanic alternative energy development, *Sargassum* harvest, military maneuvers, and oil and gas exploration and extraction. The work would also guide response/rescue of pelagic sea turtles following oil spills. Additional benefits include identification of genetic stocks and the estimation of somatic growth. Our study of the *Sargassum* drift community would allow a broad spatial and temporal description of this dynamic habitat. This information would allow calculated estimates of sea turtle density to be extrapolated across larger areas (i.e., the broader Gulf of Mexico) and longer time periods. The collection of long-term location data through satellite telemetry would allow visualization of the behavior of telemetered sea turtles in relation to the formation and dispersal of the drift habitat we observe in satellite imagery. Researchers targeting other associated species (e.g., larval fish) would receive similar benefit from the proposed seasonal and spatial descriptions of *Sargassum*.

Our research objectives address several recovery actions common to sea turtle recovery plans, for example, from the Bi-national recovery plan for the Kemp's ridley sea turtle (NMFS et al. 2011), our work would address the prioritized recovery actions:

> 122. Identify & designate marine protected areas to facilitate increased protection of important foraging, breeding, and inter-nesting habitats (Priority 1)
>
> 123. Ensure oil and gas exploration and development activities do not negatively affect foraging, breeding or interesting habitat (Priority 2)
>
> 221. Establish monitoring sites in foraging areas (Priority 1)
>
> 222. Determine migratory pathways among foraging grounds and between foraging grounds and nesting beaches (Priority 1)
>
> 226. Monitor and reduce impacts from oil/gas activities (Priority 2)
>
> 227. Monitor and reduce impacts from terrestrial and marine military activities (Priority 2)

From the loggerhead recovery plan (NMFS & USFWS 2008), our work would address the prioritized recovery actions:

> 141. Develop and maintain a comprehensive GIS database of distribution and abundance (Priority 2. This recovery action is linked to seven management actions)
>
> 142. Determine migratory pathways for all life history stages (Priority 2)
>
> 281. Assess, categorize, and map oceanic habitats (Priority 2)
>
> 29. Develop and maintain a comprehensive GIS database of neritic and oceanic habitats (used by loggerheads) and human activities that impact these habitats (Priority 2)

2821. Assess the effects of oil and gas activities on oceanic habitats used by loggerheads (Priority 3)

Federal, State and local government activities

This proposed project is part of a long-term effort to conserve sea turtles by the Florida Fish and Wildlife Conservation Commission. Related ongoing work by the Commission includes monitoring of nesting beaches, hatchling production, strandings, and in-water abundance; permitting of research and conservation projects; consultation on sea turtle management issues; and review of grant proposals.

Environmental impacts

No negative environmental, biological, social, political, or economic impacts are expected as a result of the proposed activities.

Project management

The proposed project would be administered by the Florida Fish and Wildlife Commission Fish and Wildlife Research Institute. As Principal Investigator, Blair Witherington will be the lead technical contact and project manager responsible for all oversight and implementation of the approved work plan as delineated in the proposal. Shigetomo Hirama and Robert Hardy are co-investigators who would be responsible for specific tasks in field work, data analysis, and report preparation. Curricula vitae for the investigators are listed in the attached proposal appendix. Other participants with a significant role in conducting the project include Kate Mansfield (Florida International University), who has agreed to collaborate on technical satellite tracking issues, and Chuanmin Hu (University of South Florida), who will collaborate on remote sensing data.

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Figure 1. Examples of sampling areas for surface-pelagic sea turtles in the Gulf of Mexico. Three principal sampling areas in this image of surface currents are at the eastern and northern boundaries of the Gulf Loop Current (1 and 2) and areas of persistent mesoscale eddies in the western Gulf of Mexico (3). Major surface oceanic features of interest are apparent in mean chlorophyll-a concentration (A) and sea surface temperature (B) imagery collected for the Gulf of Mexico. Satellite imagery reflects data collected during a 7-day period ending on 30 April 2012. Imagery provided by the USF Optical Oceanography Laboratory.

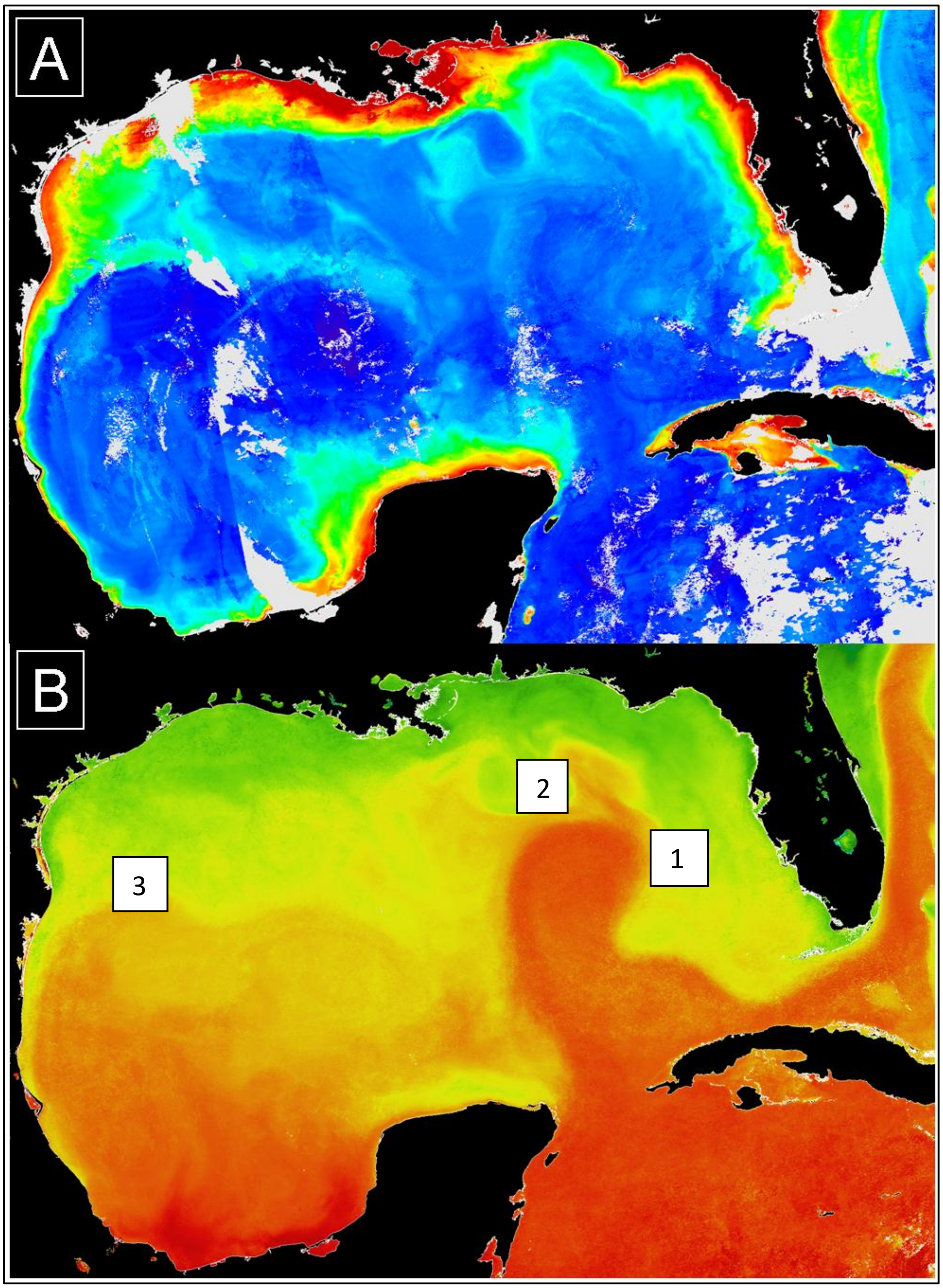

Figure 2. Examples of mesoscale eddies and converging water masses in the western Gulf of Mexico. HYCOM surface current vectors are shown above a MODIS Ocean Color Index image collected on 25 April 2012. Data provided by the USF Optical Oceanography Laboratory.

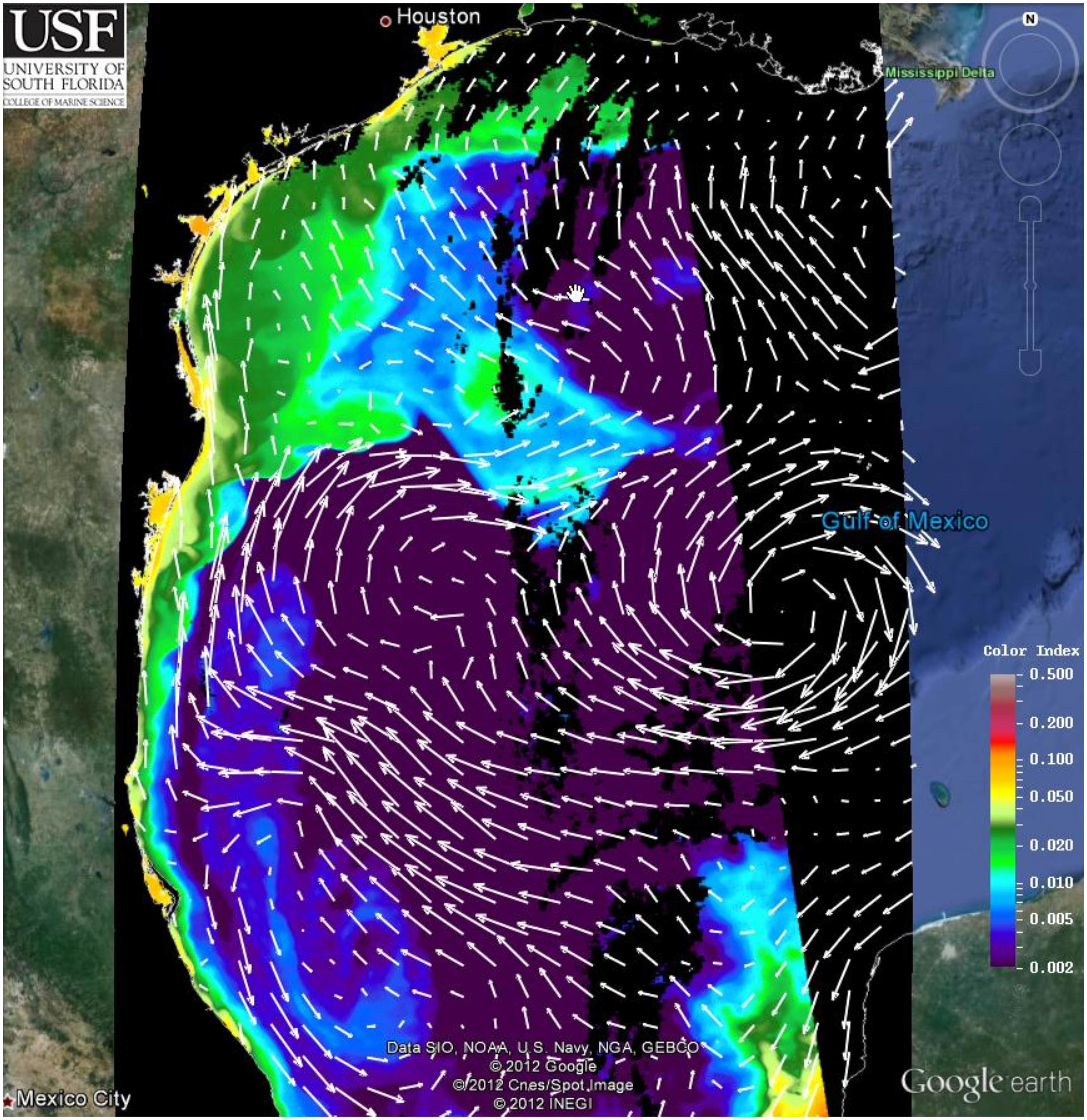

Figure 3. The dip-net capture method used for a surface-pelagic juvenile green turtle brought aboard a 15-m sportfishing vessel operating within a *Sargassum* patch in the central Gulf of Mexico.